J. Clin. Chem. Clin. Biochem.
Vol. 19, 1981, pp. 387–394

A Simultaneous Radioimmunoassay for Aldosterone and Its Precursors: Human Plasma Levels Following the Inhibition of Converting Enzyme, Before and After Blockade of Prostaglandin Biosynthesis

By *H. Witzgall*[1]) and *S. Hassan-Ali*
with the technical assistance of *B. Krischer*

Medizinische Klinik Innenstadt (Director: Prof. Dr. E. Buchborn) der Universität München

(Received March 15, 1980/February 9, 1981)

Summary: Plasma aldosterone, 18-hydroxycorticosterone, corticosterone, 18-hydroxydeoxycorticosterone, and deoxycorticosterone were determined in 15 healthy male volunteers (34 ± 2 years) following the inhibition of converting enzyme with captopril (SQ 14.225), either with or without indometacin pretreatment. These studies were performed with the aid of a radioimmunological method for the simultaneous determination of the steroids from 1.0 ml of plasma. The procedure involves one extraction and one chromatographic step. Highly sensitive and specific rabbit antisera were raised for all steroids. Precision and accuracy are shown to be equivalent to those of single steroid radioimmunoassays. Without indometacin pretreatment a constant and significant fall in aldosterone and 18-hydroxycorticosterone plasma levels was obtained after captopril. 18-Hydroxydeoxycorticosterone and corticosterone did not change after inhibition of converting enzyme. Under indometacin pretreatment all basal steroid levels were significantly reduced. After captopril, aldosterone did not change and 18-hydroxycorticosterone showed a smaller decrease than without indometacin. 18-Hydroxydeoxycorticosterone and corticosterone increased significantly 1½ hour after captopril. We conclude that indometacin significantly reduces basal mineralocorticoid production of adrenal zona glomerulosa and fasciculata. Furthermore, the effect of captopril on aldosterone and 18-hydroxycorticosterone is abolished or diminished by inhibition of prostaglandin biosynthesis, but indometacin does not prevent an augmented secretion of 18-hydroxydeoxycorticosterone and corticosterone.

Simultaner Radioimmunassay für Aldosteron und seine Vorstufen: Konzentrationen im Plasma des Menschen nach Hemmung des Converting Enzyme vor und nach Blockade der Prostaglandinbiosynthese

Zusammenfassung: Aldosteron, 18-Hydroxycorticosteron, Corticosteron, 18-Hydroxydesoxycorticosteron und Desoxycorticosteron wurden im Plasma von 15 gesunden männlichen Probanden (34 ± 2 Jahre) nach oraler Gabe des Converting Enzyme-Inhibitors Captopril (SQ 14.225) bestimmt. Die Untersuchungen sind vor und nach einer Vorbehandlung mit Indometacin durchgeführt worden. Es wird ein Radioimmunoassay beschrieben, mit dem die genannten Steroide simultan aus 1 ml Plasma bestimmt werden können. Gegen alle Steroide konnten hoch sensitive und spezifische Antiseren erzeugt werden. Die Präzision und Richtigkeit der Methode ist vergleichbar mit Radioimmunoassays, in denen nur ein Steroid bestimmt wird. Ohne Vorbehandlung mit Indometacin wurde nach Gabe von Captopril ein konstanter und signifikanter Abfall des Aldosteron und des 18-Hydroxycorticosteron im Plasma beobachtet. 18-Hydroxydesoxycorticosteron und Corticosteron änderten sich nicht nach Captopril. Nach Vorbehandlung mit Indometacin waren alle basalen Steroidkonzentrationen signifikant erniedrigt. Auf Gabe von Captopril hin änderte sich die Aldosteronkonzentration nicht und 18-Hydroxycorticosteron fiel geringer ab als ohne Vorbehandlung mit Indometacin. 18-Hydroxydesoxycorticosteron und Corticosteron stiegen unter Indometacin 1½ Stunden nach Captopril signifikant an. Die Ergebnisse zeigen, daß Indometacin die basale Mineralocorticoidsekretion der Zona glomerulosa und fasciculata supprimiert. Weiterhin wird der Effekt von Captopril auf die Aldosteron- und 18-Hydroxycorticosteronkonzentration durch die vorherige Gabe von Indometacin aufgehoben bzw. reduziert. Die Vorbehandlung mit Indometacin kann jedoch eine gesteigerte Freisetzung von 18-Hydroxydesoxycorticosteron und Corticosteron nicht verhindern.

[1]) Supported by Deutsche Forschungsgemeinschaft Wi 548/1,2.

0340-076X/81/0019-0387$02.00

Introduction

The role of prostaglandins in the regulation of aldosterone[2]) secretion is not clearly defined. It is known that corticotropin stimulates the adrenocortical prostaglandin system which, on the other hand, has been proposed to mediate corticotropin induced steroidogenesis (1–3). Furthermore, studies with intact animals have suggested that the prostaglandin system modulates the angiotensin II stimulated aldosterone production (4). However, recent attempts failed to enhance directly the aldosterone secretion by different prostaglandin metabolites in vitro (5, 6). The effect of the inhibition of the prostaglandin system on the main precursur steroids of aldosterone has not yet been evaluated in man. Plasma levels of these steroids both with and without inhibition of prostaglandin biosynthesis may provide further knowledge about regulation of aldosterone.

We developed a radioimmunoassay for simultaneous measurement of aldosterone, 18-hydroxycorticosterone, corticosterone, and deoxycorticosterone. Studies were performed in healthy men under basal conditions and after inhibition of converting enzyme with captopril (SQ 14.225; 2-O-methyl-3-mercaptopropanoyl-*L*-proline), with and without the blockade of the prostaglandin system. It could be shown that all basal steroid levels were significantly suppressed by indometacin pretreatment. Furthermore, inhibition of prostaglandin biosynthesis by indometacin pretreatment altered the response of the steroids to converting enzyme inhibition.

Materials and Methods

Radioactive chemicals

[1,2-^{3}H]18-hydroxy-11-deoxycorticosterone (specific radioactivity: 1.8 TBq/mmol, 51 Ci/mmol; 1000 counts/min = 2.8 pg), [1,2(n)-^{3}H]18-hydroxycorticosterone (1.1 TBq/mmol, 32 Ci/mmol, 1000 counts/min = 7.4 pg), [1,2(n)-^{3}H]11-deoxycorticosterone (1.2 TBq/mmol, 35 Ci/mmol; 1000 counts/min = 5.0 pg) and [1,2,6,7(n)-^{3}H]aldosterone (3.7 TBq/mmol, 102 Ci/mmol; 1000 counts/min = 2.2 pg) were purchased from Amersham Buchler, Braunschweig. [1,2,6,7(n)-^{3}H]corticosterone (3.0 TBq/mmol, 82.1 Ci/mmol; 1000 counts/min = 3.5 pg) was obtained from NEN, Langen (FRG). All radioactive chemicals were purified by thin layer chromatography before analysis.

Pharmaceuticals

Captopril (INN) (SQ 14.225; 2-O-methyl-3-mercaptopropanoyl-*L*-proline) was kindly supplied as an investigational drug by Dr. R. K. Liedke (E. R. Squibb, von Heyden GmbH, Regensburg, FRG). Indometacin (INN): Amuno®, Sharp & Dohme GmbH, München, FRG.

Chemicals and other materials

Non-radioactive steroids were purchased either from Merck, Darmstadt (FRG), Makor, Jerusalem (Israel), or Steraloids, Wilton N. H. (USA), and were stored in a desiccator before use. Tris-buffer (tris(hydroxymethyl)aminomethane), acetic acid 0.96 kg/l, benzene, dichloromethane, ethanol, isooctane, and methanol were obtained from Merck. All solvents were highly purified. Human γ-globulin (Beriglobin®) and *Freund*'s adjuvant were obtained from Behring, Marburg (FRG), charcoal (Norit A) from Serva, Heidelberg (FRG) and dextran from Fluka, Neu Ulm (FRG).

Buffer solution was 0.05 mol/l Tris buffer pH 8 (adjusted with acetic acid) and human γ-globulin 0.1 g/l. Charcoal-dextran solution consisted of 3 g/l charcoal and 1.2 g/l dextran suspended in Tris-buffer. Antisera were raised in female New Zealand white rabbits (mean age: 6 months, mean body weight: 1.2 kg).

Preparation of antigens

Antigens were synthesized according the procedure described by *Erlanger* (7).

Immunisation

The first injection consisted of 3 mg of the respective antigen dissolved in a mixture of 0.5 ml isotonic saline and 0.5 mg complete *Freund*'s adjuvant. The following injections consisted of 1 mg antigen in the same mixture and were carried out during the first three weeks at 8 day intervals, then in three weekly intervals for the subsequent booster-injections. One half of the antigen was injected intramuscularly and the other intradermally 10–15 times. Five animals were immunised for each antigen in parallel. Antisera titre was calculated as the dilution of antiserum which bound a fraction of 0.5 of the tested radioactive antigen.

Specifity

Cross reactions of the different steroids were estimated according to the method described by *Abraham* (8). Procedures were performed under assay conditions. Only biologically active C_{19} and C_{21} steroids were tested.

Affinity constant

The standard curves of the different antisera were analysed mathematically according to the method described by *Nisonoff* (9) and *Scatchard* (10).

Extraction of plasma samples

Amounts of each steroid corresponding to 8000 counts/min dissolved in a total volume of 50 µl methanol were added as internal standards to 1 ml of plasma. Plasma was extracted with 40 ml ice-cold dichloromethane in a rotating shaker after an equilibration period of 15 min. The aqueous and the organic phase were separated and, thereafter, dichloromethane was evaporated to dryness under a gentle stream of nitrogen.

Chromatography

Descending paper chromatography was performed in closed tanks. For simultaneous separation of the spectrum of steroids by one chromatographic step the system isooctane benzene methanol

---

[2]) The following trivial names are used:
Aldosterone: 18,11 hemiacetal of 11β,21-dihydroxy-3,20-dioxo-4-pregnen-18-al.
Corticosterone: 11β,21-dihydroxy-4-pregnene-3,20-dione.
Cortisone: 17,21-dihydroxy-4-pregnene-3,11,20-trione.
Cortisol: 11β,17,21-trihydroxy-4-pregnene-3,20-dione.
11-Deoxycortisol: 17,21-dihydroxy-4-pregnene-3,20-dione.
Deoxycorticosterone: 21-hydroxy-4-pregnene-3,20-dione.
Oestradiol: 1,3,5(10)-oestratriene-3,17β-diol.
18-Hydroxycorticosterone: 11β, 18,21-trihydroxy-4-pregnene-3,20-dione.
18-Hydroxydeoxycorticosterone: 18,21-dihydroxy-4-pregnene-3,20-dione.
Progesterone: 4-pregnene-3,20-dione.
Pregnenolone: 3β-hydroxy-5-pregnene-20-one.
Tetrahydroaldosterone: 3,11β,21-trihydroxy-20-oxo-5β-pregnan-18-ol.
Tetrahydrocorticosterone: 3,11β,21-trihydroxy-20-oxo-5β-pregnane.
Tetrahydrodeoxycorticosterone: 3,21-dihydroxy-20-oxo-5β-pregnane.
Testosterone: 17β-hydroxy-4-androsten-3-one.

H_2O = 60 ml + 140 ml + 120 ml + 80 ml was used. Evaluation of the procedure is described in detail elsewhere (11).

Assay procedures

All incubations were prepared in 500 µl volumes. 500 µl dextran-coated charcoal suspension was added to incubations for separating bound and free radioactivity. Only those antisera of the five steroid antigens were used which showed the highest titre at the end of the immunisation procedure. Standard curves and cross reaction analyses were performed for all steroids in buffer solution with 100 µl diluted antiserum, 100 µl of the respective radioactive steroid, 100 µl non-radioactive steroid, and 200 µl as volume substitution. For standard curves of deoxycorticosterone 200 µl buffer were replaced by 200 µl eluate from a pure paper strip to compensate for contingent unspecific blanks that might be introduced in radioimmunoassay tubes by the paper eluate containing the unknown steroid. Sensitivity analyses of deoxycorticosterone were performed in buffer solution. Preincubation was at 20 °C for 30 min with gently shaking. Incubation was at 4 °C for 24 hours.

Radioimmunoassay of aldosterone, 18-hydroxycorticosterone, 18-hydroxydeoxycorticosterone and deoxycorticosterone (unknown samples)

After eluting the respective bands from the paper strips with 10 ml methanol, the evaporated methanol specimens were dissolved in 1 ml buffer for 1 hour. 300 µl were taken for recovery analyses. 300 µl were pipetted in duplicate into radioimmunoassay tubes, together with 100 µl γ-globulin buffer (containing 1000 counts/min radioactive steroid) and 100 µl diluted antiserum.

Radioimmunoassay of corticosterone (unknown samples)

The respective band from the paper strip was eluted with 10 ml methanol. The methanol was evaporated and the residue dissolved in 2 ml buffer for 1 hour. 500 µl were taken for recovery analyses. 50 µl were pipetted in duplicate into tubes, to which the following solutions were added: 100 µl γ-globulin buffer (containing 2000 counts/min radioactive steroid), 250 µl γ-globulin buffer (volume substitution), and 100 µl diluted antiserum.

Quality-control of radioimmunoassay

For quality control purposes, two 1 ml samples of plasma from patients with bilateral adrenalectomia ("0-plasma") and two 1 ml of pool-plasma ("standard-plasma") were analysed in the same way as unknown samples in each radioimmunoassay.

Studies in human volunteers

15 healthy male volunteers aged 24 to 47 (mean 34 ± 2) years were included in the study and were fully informed of the experimental nature and the potential risks of the investigational drug. Written informed consent was obtained. Subjects were investigated under ambulatory conditions and ad libitum diet between 8 a.m. and 1 p.m. Studies were performed in active orthostasis. Blood pressure (random zero sphygmomanometer) and heart rate were recorded twice in the sitting position (2 min rest) before the oral ingestion of 100 mg captopril and ½, 1, 1½, and 3½ hours thereafter. Excretion of sodium and potassium was measured in the urine samples collected for 90 min before and for 90 min after captopril. Venous blood was drawn before, and ½, 1½, and 3½ hours after captopril. Samples were collected in heparinized tubes and were centrifuged immediately. Plasma was stored at 20 °C. The same protocol was performed again 14 days later under prostaglandin blockade with 200 mg indometacin (50 mg, respectively, at 1 a.m., 6 a.m., and at 9.30 a.m. together with captopril).

Statistical analyses

Values are mean ± S.D. for methodological values and mean ± S.E.M. for experimental results. Linear regression analyses and *Student*'s t-test were used for statistical evaluation.

Results

Evaluation of the method

Antisera

Kinetic data of the antisera are listed in table 1. Those animals were evaluated which showed the highest titre levels of the different antigens at the end of the immunisation procedure. The standard curves of the antisera (n = 10 for each steroid) showed a statistically significant difference between 0 and 3 pg ($B_o/T \pm$ S.D.) for all antisera: 18-hydroxycorticosterone, aldosterone, corticosterone and deoxycorticosterone ($p < 0.01$). Thus, 3 pg of each steroid could be distinguished from zero by the respective antisera (sensitivity). All antisera were tested against at least 12 biologically relevant steroids (specificity). Cross-reactivity of the different antisera are shown in table 2.

Extraction and chromatography

The following radioactivity recovery rates for the five steroids were obtained after extraction and chromatography (n = 10): 18-hydroxycorticosterone 39 ± 7, aldosterone 37 ± 7, 18-hydroxydeoxycorticosterone 38 ± 5, corticosterone 40 ± 6, and deoxycorticosterone 50 ± 8%.

Assay parameters

Precision

Within assay variation was determined from ten replicate measurements in the same assay of samples from a plasma pool (intraassay). The following values were found (coefficient of variation in parenthesis): 18-hydroxycorticosterone 231 ± 29 (12.5%), aldosterone 146 ± 13 (8.9%), 18-hydroxydeoxycorticosterone 112 ± 11 (9.8%) and deoxycorticosterone 196 ± 21 ng/l (10.7%); corticosterone 5.04 ± 0.37 µg/l (7.3%). Between assay variation was evaluated from samples of the same plasma pool determined in five different assays (interassay): 18-hydroxycorticosterone 258 ± 38 (14.7%), aldosterone 137 ± 15 (10.9%), 18-hydroxydeoxycorticosterone

Tab. 1. Comparison of kinetic data of the used antisera. K_D = Affinity constant.

Antisera against	Characteristics of the antisera: Final titer	K_D (mol/l)	Number of booster injections
18-Hydroxycorticosterone	1: 3500	2.3×10^{-10}	4
Aldosterone	1:150000	1.1×10^{-10}	6
18-Hydroxydeoxycorticosterone	1: 70000	1.6×10^{-10}	6
Corticosterone	1: 25000	1.6×10^{-10}	8
Deoxycorticosterone	1: 30000	1.6×10^{-10}	6

Tab. 2. Cross-reactivity in per cent of the tested antisera with different biologically active steroids.

Cross-reacting Steroid	18-Hydroxy-corticosterone	Aldosterone	18-Hydroxy-deoxy-corticosterone	Cortico-sterone	Deoxycortico-sterone
18-Hydroxycorticosterone	100.0	0.142	3.300	0.255	0.370
Aldosterone	0.043	100.0	3.700	0.013	0.153
18-Hydroxydeoxycorticosterone	0.114	0.006	100.0	0.065	0.694
Corticosterone	0.048	0.153	0.021	100.0	0.024
Deoxycorticosterone	0.013	0.444	0.051	0.072	100.0
Cortisone	0.006	0.923	0.031	0.058	0.049
Cortisol	0.034	0.333	0.015	0.061	0.068
Testosterone	0.025	0.240	0.062	0.176	0.038
Progesterone	0.009	0.571	0.357	0.040	0.036
17β-Oestradiol	0.013	0.007	0.015	0.035	0.014
11-Deoxycortisol	0.016	-	-	0.027	0.083
Pregnenolone	0.018	-	0.053	0.111	0.462
Tetrahydroaldosterone	0.016	0.800	0.025	0.017	-
Tetrahydrocorticosterone	-	0.070	-	-	-
Tetrahydrodeoxycorticosterone	-	0.034	-	-	-

128 ± 15 (11.7%) and deoxycorticosterone 201 ± 28 ng/l (13.9%); corticosterone 4.99 ± 0.47 µg/l (9.4%).

Accuracy

To test the accuracy of the multi-steroid assay, known quantities of unlabelled steroids were added to 1 ml of pool plasma taken from patients with *Addison*'s diseases. Samples were examined by replicate analyses of each point (n = 10). Increasing amounts of steroids were added in physiological concentrations. In figure 1 measured steroid concentrations are plotted against the amounts of steroid added to the plasma.

Blanks

Assay blanks were evaluated from 1 ml plasma of a bilaterally adrenalectomized patient and were included in all procedures of the assay (n = 10): aldosterone 7 ± 3, 18-hydroxycorticosterone 8 ± 5, 18-hydroxydeoxycorticosterone 6 ± 3 ng/l, and corticosterone 0.11 ± 0.04 µg/l. For deoxycorticosterone an assay blank of 42 ± 13 ng/l steroid-free plasma was found.

Paper blanks were tested by replacing buffer solution with equal amounts of paper eluate in all tubes of the standard curves (fig. 2). In the paper eluate curve of corticosterone, bound radioactivity of all points was slightly higher than in the normal standard curve ("negative blank"). Bound radioactivity in the deoxycorticosterone standard curve was higher than in the paper eluate curve which is consistent with a paper blank of about 10 pg for all points determined.

Practicability

During five working days a skillful technician can determine the five different steroids in 25 plasma samples, which represents 125 steroid values per week. Calculation of the standard curves and the plasma samples is performed by electronic data processing (spline).

Experimental protocol

Response to captopril without indometacin pretreatment

Heart rate before captopril ingestion was 76 ± 2 min^{-1} and remained constant during the investigation. Mean arterial blood pressure fell continuously from the basal value before captopril treatment (97 ± 3) to a minimum 1 hour thereafter (87 ± 2 mmHg; $p < 0.02$) and then increased again to pretreatment levels 3½ hours after captopril. Basal sodium (206 ± 23) and potassium (107 ± 14 µmol/min) excretion remained unchanged after captopril. A continuous and significant decrease of aldosterone and 18-hydroxydeoxycorticosterone was found after inhibition of converting enzyme. 18-Hydroxydeoxycorticosterone and corticosterone, however, remained unchanged (fig. 3). Basal deoxycorticosterone level was 171 ± 9 ng/l.

Response to captopril under indometacin pretreatment

All values for heart rate (basal 70 ± 2 min^{-1}) were significantly ($p < 0.05$) and constantly lower than those without indometacin pretreatment. Mean basal blood pressure on indometacin (97 ± 3) was unchanged in comparison with the control. However, the decrease of blood pressure 1 hour after captopril (93 ± 2 mmHg) was significantly smaller ($p < 0.05$) than in the experiment without indometacin pretreatment. Basal sodium (201 ± 28) and potassium (103 ± 13 µmol/min) excretion rates under indometacin remained unchanged before and after captopril.

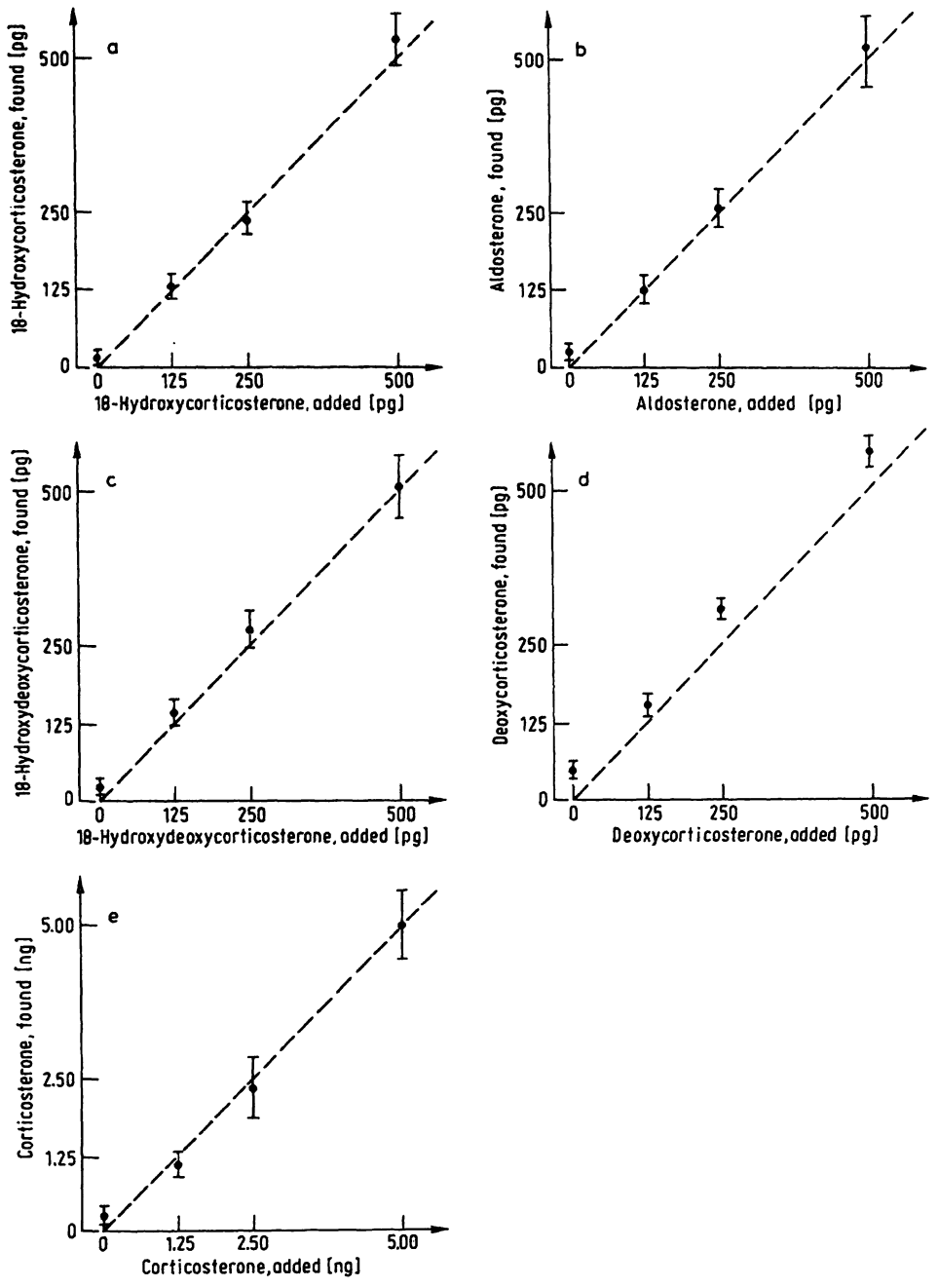

Fig. 1. Correlations between added and estimated amounts of unlabelled steroid:

a) 18-hydroxycorticosterone:	y = 5 + 1.02 x;	r = 0.98
b) aldosterone:	y = 12 + 1.01 x;	r = 0.98
c) 18-hydroxydeoxycorticosterone:	y = 25 + 0.96 x;	r = 0.98
d) deoxycorticosterone:	y = 48 + 1.02 x;	r = 0.99
e) corticosterone:	y = 0.05 + 0.99 x;	r = 0.98
r = coefficient of correlation.	n = 10.	

All control steroid levels in active orthostasis were significantly lower under prostaglandin biosynthesis inhibition. Basal deoxycorticosterone level was 119 ± 28 ng/l ($p < 0.05$). Aldosterone did not change after captopril. 18-Hydroxycorticosterone remained constant ½ hour after captopril but showed a significant decrease 1½ and 3½ hours after converting enzyme inhibition. However, 18-hydroxydeoxycorticosterone and corticosterone levels increased significantly 1½ hour after captopril (fig. 3).

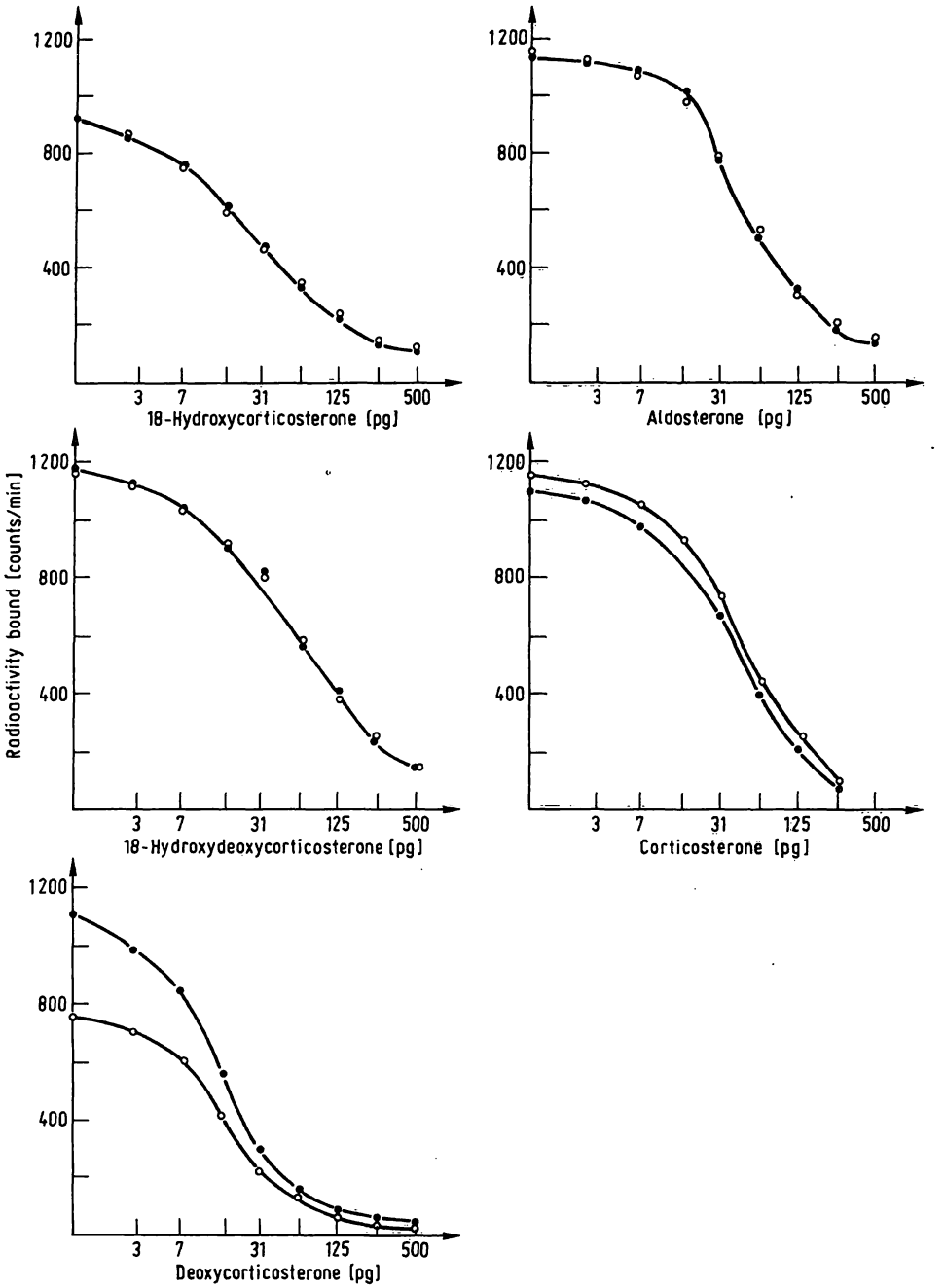

Fig. 2. Standard curves of the spectrum of steroid antisera under normal conditions (closed circles) compared with standard curves in which buffer solution was replaced by paper eluate (open circles).

Discussion

A highly specific and sensitive radioimmunoassay is described for aldosterone and its precursors 18-hydroxycorticosterone, corticosterone, deoxycorticosterone and 18-hydroxydeoxycorticosterone from 1 ml plasma. The obvious advantage of the presented method is that very low concentrated steroids can be simultaneously estimated from a minute amount of human plasma by a simplified procedure. Specificity of the evaluated antisera was assessed by testing biologically relevant cross-reacting corticosteroids. Cross-reactions were comparable with data recently reported (12–15). No gross cross-reactivity was detected in any of the antisera. Sensitivity of the different standard curves allows the measurement of very small amounts of the respective steroid. The calculated affinity constants of the antisera are comparable to values recently reported (14, 16, 17). Recovery rates after extraction and chromatography were in the range of 40–50% for the spectrum of mineralocorticoids. This high yield was achieved by using only *one* extraction- and *one* chromatographic step.

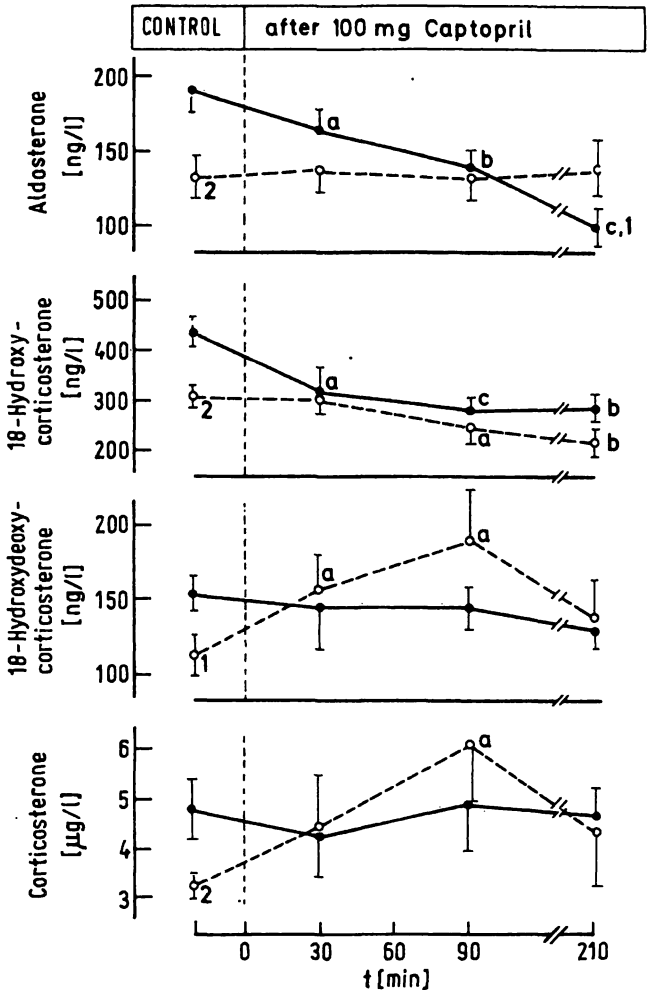

Fig. 3. Concentrations of plasma aldosterone, 18-hydroxycorticosterone, 18-hydroxydeoxycorticosterone, and corticosterone before, 30, 90, and 210 min after captopril. Determinations were performed both without (●——●) and under (o----o) indometacin pretreatment.
a) $p < 0.05$, b) $p < 0.01$, and c) $p < 0.005$ vs. respective control level.
1) $p < 0.05$ and 2) $p < 0.01$ vs. without indometacin.

Preparation and determination of 18-hydroxylated mineralocorticoids are difficult because of their known instability in acidified solutions (18, 19). In order to avoid formation of various compounds by reactions with the 18-hydroxyl-group, we therefore used highly purified organic solvents without any traces of acids (Uvasol®) and all buffer solutions were alkaline (pH 8). So far, paper chromatography guarantees the most careful handling of 18-hydroxylated steroids compared with other chromatographic methods (e.g. thin layer or column chromatography with silica gel). In this context exposure to organic solvents is shortened by the described assay. In recently published multiple assays at least two extractions and chromatographic separations were necessary before radioimmunoassay (20–22).

For aldosterone, 18-hydroxycorticosterone, 18-hydroxydeoxycorticosterone, and corticosterone no relevant assay blanks could be detected. The blanks evaluated from plasma of a patient with bilateral adrenalectomy were found to be below the sensitivity of the respective standard curves. This was consistent with the result, that no paper blanks of those steroids could be demonstrated. The deoxycorticosterone assay blank of about 40 ng/l was mainly due to the paper blank of this steroid, which covered the complete standard curve. Several attempts failed to eliminate or reduce the paper blank of this assay. Blank-free conditions could only be established by displacing buffer solution with paper eluate for constructing the standard curve (23). By this manipulation, the assay sensitivity of deoxycorticosterone was considerably reduced.

Standard curves of our assay were evaluated with a small amount of tracer (2000 counts/min). Depending on the specific radioactivity of the different steroids, the quantity added to the antigen in the respective radioimmunoassay tube varied from a minimal 4.4 pg (aldosterone) to a maximal 14.8 pg (18-hydroxycorticosterone). This is of great importance with respect to the low concentrations of steroids (mean plasma concentration: 50–200 ng/l). Assays for these steroids were so constructed that tracer amounts used for recovery analyses of samples after extraction and chromatography could partly be taken for radioimmunoassay (about 1000 counts/min for 40% total recovery). Therefore only 1000 counts/min must be added to assay tubes to fit the 2000 counts/min of the standard curves (24). The methodological procedure needs a very careful and accurate handling of all samples to provide constant recovery rates.

Studies in human volunteers

Studies were performed in normotensive volunteers under ambulatory conditions (active orthostasis) without any instructions for diet in the sodium replete state. Captopril induced a sustained fall in aldosterone (25) and 18-hydroxycorticosterone without indometacin pretreatment. Corticosterone and 18-hydroxydeoxycorticosterone remained unchanged after captopril. Inhibition of the conversion of angiotensin I to angiotensin II by captopril is the most probable reason for the fall in aldosterone and 18-hydroxycorticosterone. This may support the hypothesis that aldosterone and 18-hydroxycorticosterone are more sensitive to changes of angiotensin II levels than corticosterone and 18-hydroxydeoxycorticosterone which are mainly regulated by corticotropin (21, 26). The inhibition of prostaglandin biosynthesis by indometacin was accompanied by a significant decrease of all control steroid levels during active orthostasis, which may be due to the inhibition of the adrenocortical prostaglandin system. On the other hand, intrarenal prostaglandins stimulate renin release (27). Inhibition of renal prostaglandin formation reduces renin secretion, which results in diminished basal angiotensin II plasma levels under indometacin.

This mechanism, in addition, may further decrease aldosterone and 18-hydroxycorticosterone levels. Basal plasma levels of aldosterone and 18-hydroxycorticosterone under control conditions were greatly reduced by indometacin, and no or only a smaller decrease was found after captopril without indometacin pretreatment.

Under inhibition of prostaglandin biosynthesis a significant increase of corticosterone and 18-hydroxydeoxycorticosterone was found 1½ hour after captopril. The reason for this behaviour remained unresolved. Two possible mechanisms should be discussed:

(1) Since corticosterone and 18-hydroxydeoxycorticosterone are mainly regulated by corticotropin, an augmented corticotropin secretion could be responsible: Under indometacin, vasodilating vascular prostaglandins are diminished, thus resulting in an increased peripheral vascular resistance (28). Maintenance of control blood pressure was achieved by a compensatory reduction of heart rate. In this labile haemodynamic equilibrium the application of the blood pressure lowering drug, captopril, may be a prominent stress stimulus for the organism, which might consequently contribute to an enhanced corticotropin secretion;

(2) On the other hand, an elevated sensitivity and/or number of corticotropin receptors on zona fasciculata cells must be considered. Recently it has been shown that the inhibition of the prostaglandin system effected an increase of β-adrenoreceptors on human leukocyte membranes (29). A similar mechanism for corticotropin receptors on the level of zona fasculata cells could explain our findings.

The data of our experiments suggest that inhibition of adrenocortical prostaglandin biosynthesis by indometacin decreased all basal steroid levels, but could not prevent an augmented secretion of corticosterone and 18-hydroxydeoxycorticosterone after captopril administration.

Acknowledgements

The authors wish to thank
Dres *B. Penke* and *J. Varga*, Budapest, Hungary, for synthesizing aldosterone-, corticosterone-, and 18-hydroxydeoxycorticosterone antigens;
Dr. *R. K. Liedtke* (E. R. Squibb, von Heyden, Regensburg, FRG) for supplying captopril.

References

1. Saruta, T. & Kaplan, N. M. (1972), J. Clin. Invest. *51*, 2246–2251.
2. Honn, V. K. & Chavin, W. (1976), Biochem. Biophys. Res. Comm. *72*, 1319–1326.
3. Laychock, S. G. & Rubin, R. P. (1975), Prostaglandins *10*, 529–540.
4. Campbell, W. B., Gomez-Sanchez, C. E., Adams, B. V., Schmitz, J. M. & Itskovitz, H. D. (1979), J. Clin. Invest. *64*, 1552–1557.
5. Laychock, S. G. & Walker, L. (1979), Prostaglandins *18*, 793–809.
6. Matsuoka, H., Tan, S. Y. & Mulrow, P. J. (1980), Prostaglandins *19*, 291–298.
7. Erlanger, B., Borck, F., Beiser, S. M. & Liebermann, S. (1957), J. Biol. Chem. *228*, 713–721.
8. Abraham, G. E. (1969), J. Clin. Endocrinol. *29*, 866–875.
9. Nisonoff, A. & Pressman, D. (1957), J. Immunol. *80*, 417–423.
10. Scatchard, G. (1949), Ann. N. Y. Acad. Sci. *51*, 660–669.
11. Witzgall, H. & Hassan-Ali, S. (1980), J. Chromatography *198*, 70–75.
12. Martin, V. J., Edwards, C. R. W., Biglieri, E. G., Vinson, G. P. & Bartter, F. C. (1975), Steroids *26*, 591–604.
13. Vecsei, P., Penke, B. & Joumaah, A. (1972), Experientia *28*, 622–628.
14. Hornung, J., Gless, K. H., Abdelhamid, S., Vielhauer, W. & Vecsei, P. (1978), Clin. Chim. Acta *87*, 181–188.
15. Castro, A., Bartos, D., Jelen, B. & Kutas, M. (1973), Steroids *22*, 851–858.
16. Sulon, J., Palem-Vliers, M., Genard, P., Cittanova, N. & Desfosses, B. (1976), Clin. Chim. Acta *72*, 275–284.
17. Sancho, D. & Haber, E. (1978), J. Clin. Endocrinol. Metab. *47*, 391–399.
18. Roy, A. K., Ramirez, L. C. & Ulick, S. (1976), J. Steroid Biochem. *7*, 81–87.
19. Aragones, A., Gros, E. G., Lantos, C. P. & Locascio, G. A. (1978), J. Steroid Biochem. *9*, 175–180.
20. Mason, P. A. & Fraser, R. (1975), J. Endocrinol. *64*, 277–288.
21. Schöneshöfer, M. (1977), J. Steroid Biochem. *8*, 995–1009.
22. Sippell, W. G., Bidlingmaier, F., Becker, H., Brüssing, T., Dörr, H., Hahn, H., Golder, W., Hollmann, G. & Knorr, D. (1978), J. Steroid Biochem. *9*, 63–74.
23. Schöneshöfer, M., Oelkers, W. & Harendt, H. (1975), J. Clin. Chem. Clin. Biochem. *13*, 143–147.
24. Klumpp, F., Rössler, R. & Klaus, D. (1974), J. Clin. Chem. Clin. Biochem. *12*, 128–134.
25. Johns, D. W., Baker, K. M., Ayers, C. R., Vaughan, E. D., Carey, R. M., Peach, M. J., Yancey, M. R., Ortt, E. M. & Williams, S. C. (1980), Hypertension, *2*, 567–575.
26. Melby, J. C., Dole, S. L., Grekin, R. J., Gaunt, R. & Wilson, T. E. (1972), Recent Progr. Hormon. Res. *28*, 287–351.
27. Weber, P. C., Larsson, C., Anggard, E., Hamberg, M., Corey, E. J., Nicolaou, K. C. & Sammelsson, B. (1976), Circ. Res. *39*, 868–874.
28. Wennmalm, A. (1978), Clin. Sci. Mol. Med. *54*, 141–148.
29. Güllner, H.G., Kafka, M. S. & Bartter, F. C. (1980), Clin. Sci. *59*, 397–400.

Dr. med. Helmut Witzgall
Medizinische Klinik Innenstadt
Ziemssenstr. 1a
D-8000 München 2